REMARKS

To assist in the examination of this application and as required by 37 CFR 1.121, enclosed herewith as Appendix 1 is a marked up version of the changes made to the specification to indicate how the previous version of the specification has been modified to produce the clean replacement paragraphs. The modifications are indicated by underlining and in bold type for additions, and by strikeouts for deletions.

As indicated above, the Specification of the instant application has been amended to make changes necessitated by the substitution of the drawings to conform with the margin requirements of 35 CFR 1.84(g) and to include the Sequence Listing. Accompanying this Preliminary Amendment is the paper copy of the Sequence Listing and a Request to Use Computer Readable Form from Another Application.

In accordance with 37 CFR Section 1.821(g), the undersigned Attorney for the Applicants states that the Sequence Listing includes no new matter.

CONCLUSION

Applicants respectfully submit that the above amendments do not add any new matter and request that they be entered for the instant application. If the undersigned

Attorney for the Applicants can be of any assistance in regard to this Preliminary Amendment, she can be reached at (415) 981-2034.

Respectfully submitted,

Leona L. Lauder
Attorney for Applicants
Registration No. 30,863

Dated: June 14, 2001

APPENDIX 1

The paragraph on page 4, lines 5-11 has been amended as follows:

Herein disclosed is the MN gene, a cellular gene which is the endogenous component of the MaTu agent. A full-length cDNA sequence for the MN gene is shown in ~~Figure 1~~ **Figures 1A-1C** [SEQ. ID. NO.: 1]. ~~Figure 15a-d provides~~ **Figures 15A-15F provide** a complete genomic sequence for MN [SEQ. ID. NO.: 5]. Figure 25 provides the sequence for a proposed MN promoter region [SEQ. ID. NO.: 27].

The paragraph on page 5, lines 18-26 has been amended as follows:

Further, such isolated nucleic acids that encode MN proteins or polypeptides can also include the MN nucleic acids of the genomic clone shown in ~~Figure 15a-d~~ **Figures 15A-15F**, that is, SEQ. ID. NO.: 5, as well as sequences that hybridize to it or its complement under stringent conditions, or would hybridize to SEQ. ID. NO.: 5 or to its complement under such conditions, but for the degeneracy of the genetic code. Degenerate variants of SEQ. ID. NOS.: 1 and 5 are within the scope of the invention.

The paragraph on page 6, lines 11-12 has been amended as follows:

(a) a nucleic acid having the nucleotide sequence shown in ~~Figure 15a-d~~ **Figures 15A-15F** [SEQ. ID. NO.: 5] and its complement;

The paragraph on page 9, lines 10-23 has been amended as follows:

In HeLa and in tumorigenic HeLa x fibroblast hybrid (H/F-T) cells, MN protein is manifested as a "twin" protein p54/58N; it is glycosylated and forms disulfide-linked oligomers. As determined by electrophoresis upon reducing gels, MN proteins have molecular weights in the range of from about 40 kd to about 70 kd, preferably from about 45 kd to about 65 kd, more preferably from about 48 kd to about 58 kd. Upon non-reducing gels, MN proteins in the form of oligomers have molecular weights in the range of from about 145 kd to about 160 kd, preferably from about 150 to about 155 kd, still more preferably from about 152 to about 154 kd. A predicted amino acid sequence for a preferred MN protein of this invention is shown in ~~Figure 1~~ **Figures 1A-1C** [SEQ. ID. NO. 2].

The paragraph at page 12, lines 6-23 has been amended as follows:

The invention further is directed to MN-specific antibodies, which can be used diagnostically/prognostically and may be used therapeutically. Preferred according to this

invention are MN-specific antibodies reactive with the epitopes represented respectively by the amino acid sequences of the MN protein shown in ~~Figure 1~~ **Figures 1A-1C** as follows: from AA 62 to AA 67 [SEQ. ID. NO.: 10]; from AA 55 to AA 60 [SEQ. ID. NO.: 11]; from AA 127 to AA 147 [SEQ. ID. NO.: 12]; from AA 36 to AA 51 [SEQ. ID. NO.: 13]; from AA 68 to AA 91 [SEQ. ID. NO.: 14]; from AA 279 to AA 291 [SEQ. ID. NO.: 15]; and from AA 435 to AA 450 [SEQ. ID. NO.: 16]. More preferred are antibodies reactive with epitopes represented by SEQ. ID. NOS.: 10, 11 and 12. Still more preferred are antibodies reactive with the epitopes represented by SEQ. ID NOS: 10 and 11, as for example, respectively Mabs M75 and MN12. Most preferred are monoclonal antibodies reactive with the epitope represented by SEQ. ID. NO.: 10.

The paragraph at page 15, lines 4-14 has been amended as follows:

This invention also concerns methods of treating neoplastic disease and/or pre-neoplastic disease comprising inhibiting the expression of MN genes by administering antisense nucleic acid sequences that are substantially complementary to mRNA transcribed from MN genes. Said antisense nucleic acid sequences are those that hybridize to such mRNA under stringent hybridization conditions. Preferred are antisense nucleic acid

sequences that are substantially complementary to sequences at the 5' end of the MN cDNA sequence shown in ~~Figure 1~~ **Figures 1A-1C**. Preferably said antisense nucleic acid sequences are oligonucleotides.

The paragraph on page 23, lines 3-9 has been amended as follows:

There are twenty main amino acids, each of which is specified by a different arrangement of three adjacent nucleotides (triplet code or codon), and which are linked together in a specific order to form a characteristic protein. A three-letter or one-letter convention is used herein to identify said amino acids, as, for example, in ~~Figure 1~~ **Figures 1A-1C** as follows:

The paragraph on page 24, lines 8-11 has been amended as follows:

~~Figure 1 provides~~ **Figures 1A-1C provide** the nucleotide sequence for a full-length MN cDNA [SEQ. ID. NO.: 1] clone isolated as described herein. ~~Figure 1 also sets~~ **Figures 1A-1C also set** forth the predicted amino acid sequence [SEQ. ID. NO.: 2] encoded by the cDNA.

The paragraph on page 26, lines 17-24 has been amended as follows:

~~Figure 11~~ **Figures 11A and 11B** (discussed in Example 8) graphically ~~illustrates~~ **illustrate** the results from radioimmunoprecipitation experiments with ^{125}I-GEX-3X-MN protein and different antibodies. The radioactive protein (15 x 10^3 cpm/tube) was precipitated with ascitic fluid or sera and SAC as follows: (A) ascites with MAb M75; (B) rabbit anti-MaTu serum; (C) normal rabbit serum; (D) human serum L8; (E) human serum KH; and (F) human serum M7.

The paragraph on page 28, lines 5-8 has been amended as follows:

~~Figure 15a-d provides~~ **Figures 15A-15F provide** a 10,898 bp complete genomic sequence of MN [SEQ. ID. NO.: 5]. The base count is as follows: 2654 A; 2739 C; 2645 G; and 2859 T. The 11 exons are shown in capital letters.

The paragraph on page 30, lines 21-23 has been amended as follows:

~~Figure 23 illustrates~~ **Figure 23A-1 to 23C illustrate** flow cytometric analyses of asynchronous cell populations of control and MN cDNA-transfected NIH 3T3 cells.

The two paragraphs on page 36, lines 7-24 have been amended as follows:

Examples herein show that MX and MN are two different entities, that can exist independently of each other. MX (LCMV) as an exogenous, transmissible agent can multiply in fibroblasts and in H/F-N hybrid cells which are not expressing MN-related proteins ~~(Figure 6)~~ **(Figures 6A and 6B)**. In such cells, MX does not induce the production of MN protein. MN protein can be produced in HeLa and other tumor cells even in the absence of MX as shown in Figures 6-9. However, MX is a potent inducer of MN-related protein in HeLa cells; it increases its production thirty times over the concentration observed in uninfected cells (Figures 7 and 12, Table 2 in Example 8, below).

MN Gene--Cloning and Sequencing

~~Figure 1 provides~~ **Figures 1A-1C provide** the nucleotide sequence for a full-length MN cDNA clone isolated as described below [SEQ. ID. NO.: 1]. ~~Figure 15a-d provides~~ **Figures 15A-15F provide** a complete MN genomic sequence [SEQ. ID. NO.: 5]. Figure 25 shows the nucleotide sequence for a proposed MN promoter [SEQ. ID. NO.: 27].

The paragraph beginning on page 37, line 12 to page 38, line 9 has been amended as follows:

It is further understood that the nucleotide sequences herein described and shown in Figures ~~1, 15a-d~~ **1A-1C, 15A-15F** and

25, represent only the precise structures of the cDNA, genomic and promoter nucleotide sequences isolated and described herein. It is expected that slightly modified nucleotide sequences will be found or can be modified by techniques known in the art to code for substantially similar or homologous MN proteins and polypeptides, for example, those having similar epitopes, and such nucleotide sequences and proteins/polypeptides are considered to be equivalents for the purpose of this invention. DNA or RNA having equivalent codons is considered within the scope of the invention, as are synthetic nucleic acid sequences that encode proteins/polypeptides homologous or substantially homologous to MN proteins/polypeptides, as well as those nucleic acid sequences that would hybridize to said exemplary sequences [SEQ. ID. NOS. 1, 5 and 27] under stringent conditions or that but for the degeneracy of the genetic code would hybridize to said cDNA nucleotide sequences under stringent hybridization conditions. Modifications and variations of nucleic acid sequences as indicated herein are considered to result in sequences that are substantially the same as the exemplary MN sequences and fragments thereof.

The paragraph on page 40, lines 2-10 has been amended as follows:

Attempts to isolate a full-length clone from the original cDNA library failed. Therefore, we performed a rapid amplification of cDNA ends (RACE) using MN-specific primers, R1 and R2, derived from the 5' region of the original cDNA clone. The RACE product was inserted into pBluescript, and the entire population of recombinant plasmids was sequenced with an MN-specific primer ODN1. In that way, we obtained a reliable sequence at the very 5' end of the MN cDNA as shown in ~~Figure 1~~ **Figures 1A-1C** [SEQ. ID. NO.: 1].

Table 1 on page 45, lines 1-30 has been amended as follows:

TABLE 1

Exon-Intron Structure of the Human MN Gene

Exon	Size	Genomic Position**	SEQ ID NO	5'splice donor	SEQ ID No
1	445	*3507-3951	28	AGAAG gtaagt	67
2	30	5126-5155	29	TGGAG gtgaga	68
3	171	5349-5519	30	CAGTC gtgagg	69
4	143	5651-5793	31	CCGAG gtgagc	70
5	93	5883-5975	32	TGGAG gtacca	71
6	67	7376-7442	33	GGAAG gtcagt	72
7	158	8777-8934	34	AGCAG gtgggc	73
8	145	9447-9591	35	GCCAG gtacag	74
9	27	9706-9732	36	TGCTG gtgagt	75
10	82	10350-10431	37	CACAG gtatta	76
11	191	10562-10752	38	ATAAT end	

Intron	Size	Genomic Position**	SEQ ID NO	3'splice acceptor	SEQ ID NO
1	1174	3952-5125	39	atacag GGGAT	77
2	193	5156-5348	40	ccccag GCGAC	78
3	131	5520-5650	41	acgcag TGCAA	79
4	89	5794-5882	42	tttcag ATCCA	80
5	1400	5976-7375	43	ccccag GAGGG	81
6	1334	7443-8776	44	tcacag GCTCA	82
7	512	8935-9446	45	ccctag CTCCA	83
8	114	9592-9705	46	ctccag TCCAG	84
9	617	9733-10349	47	tcgcag GTGACA	85
10	130	10432-10561	48	acacag AAGGG	86

** positions are related to nt numbering in whole genomic sequence including the 5' flanking region ~~[Figure 15a-d]~~ **[Figures 15A-15F]**

* number corresponds to transcription initiation site determined below by RNase protection assay

The two paragraphs beginning on page 55, line 2 to page 56, line 6 have been amended as follows:

The ORF of the MN cDNA shown in ~~Figure 1 has~~ **Figures 1A-1C have** the coding capacity for a 459 amino acid protein with a calculated molecular weight of 49.7 kd. MN protein has an estimated pI of about 4. As assessed by amino acid sequence analysis, the deduced primary structure of the MN protein can be divided into four distinct regions. The initial hydrophobic region of 37 amino acids (AA) corresponds to a signal peptide. The mature protein has an N-terminal part of 377 AA, a hydrophobic transmembrane segment of 20 AA and a C-terminal region of 25 AA. Alternatively, the MN protein can be viewed as having five domains as follows: (1) a signal peptide [amino acids (AA) 1-37; SEQ. ID. NO.: 6]; (2) a region of homology to collagen alpha1 chain (AA 38-135; SEQ. ID. NO.: 50); (3) a carbonic anhydrase domain (AA 136-391; SEQ. ID. NO.: 51); (4) a transmembrane region (AA 414-433; SEQ. ID. NO.: 52); and (5) an intracellular C terminus (AA 435-459; SEQ. ID. NO.: 53). (The AA numbers are keyed to ~~Figure 1~~ **Figures 1A-1C**.)

More detailed insight into MN protein primary structure disclosed the presence of several consensus sequences. One potential N-glycosylation site was found at position 346 of ~~Figure 1~~ **Figures 1A-1C**. That feature, together with a predicted membrane-spanning region are consistent with the results, in

which MN was shown to be an N-glycosylated protein localized in the plasma membrane. MN protein sequence deduced from cDNA was also found to contain seven S/TPXX sequence elements [SEQ. ID. NOS.: 25 AND 26] (one of them is in the signal peptide) defined by Suzuki, J. Mol. Biol., 207: 61-84 (1989) as motifs frequently found in gene regulatory proteins. However, only two of them are composed of the suggested consensus amino acids.

The two paragraphs beginning on page 57, line 6 to page 58, line 2 have been amended as follows:

The MN gene was found to clearly be a novel sequence derived from the human genome. Searches for amino acid sequence similarities in protein databases revealed as the closest homology a level of sequence identity (38.9% in 256 AA or 44% in an 170 AA overlap) between the central part of the MN protein [AAs 136-391 (SEQ. ID. NO: 51)] or 221-390 [SEQ. ID. NO.: 54] of ~~Figure 1~~ **Figures 1A-1C** and carbonic anhydrases (CA). However, the overall sequence homology between the cDNA MN sequence and cDNA sequences encoding different CA isoenzymes is in a homology range of 48-50% which is considered by ones in the art to be low. Therefore, the MN cDNA sequence is not closely related to any CA cDNA sequences.

Only very closely related nt sequences having a homology of at least 80-90% would hybridize to each other under

stringent conditions. A sequence comparison of the MN cDNA sequence shown in ~~Figure 1~~ **Figures 1A-1C** and a corresponding cDNA of the human carbonic anhydrase II (CA II) showed that there are no stretches of identity between the two sequences that would be long enough to allow for a segment of the CA II cDNA sequence having 50 or more nucleotides to hybridize under stringent hybridization conditions to the MN cDNA or vice versa.

The two paragraphs beginning on page 59, line 18 to page 60, line 4 have been amended as follows:

The phrase "MN proteins and/or polypeptides" (MN proteins/polypeptides) is herein defined to mean proteins and/or polypeptides encoded by an MN gene or fragments thereof. An exemplary and preferred MN protein according to this invention has the deduced amino acid sequence shown in ~~Figure 1~~ **Figures 1A-1C**. Preferred MN proteins/polypeptides are those proteins and/or polypeptides that have substantial homology with the MN protein shown in ~~Figure 1~~ **Figures 1A-1C**. For example, such substantially homologous MN proteins/polypeptides are those that are reactive with the MN-specific antibodies of this invention, preferably the Mabs M75, MN12, MN9 and MN7 or their equivalents.

The paragraph on page 62, lines 4-13 has been amended as follows:

A representative method to prepare the MN proteins shown in ~~Figure 1~~ **Figures 1A-1C** or fragments thereof would be to insert the full-length or an appropriate fragment of MN cDNA into an appropriate expression vector as exemplified below. The fusion protein GEX-3X-MN expressed from XL1-Blue cells is nonglycosylated. Representative of a glycosylated, recombinantly produced MN protein is the MN 20-19 protein expressed from insect cells. The MN 20-19 protein was also expressed in a nonglycosylated form in E. coli using the expression plasmid pEt-22b [Novagen].

The paragraph beginning on page 69, line 13 to page 70, line 3 has been amended as follows

Another representative, recombinantly produced MN protein of this invention is the MN 20-19 protein which, when produced in baculovirus-infected Sf9 cells [Spodoptera frugiperda cells; Clontech; Palo Alto, CA (USA)], is glycosylated. The MN 20-19 protein misses the putative signal peptide (AAs 1-37) of SEQ. ID. NO.: 6 ~~(Figure 1)~~ **(Figures 1A-1C)**, has a methionine (Met) at the N-terminus for expression, and a Leu-Glu-His-His-His-His-His-His [SEQ. ID NO.: 22] added to the C-terminus for purification. In order to insert the portion of the MN coding sequence for the GEX-3X-MN fusion protein into alternate expression systems, a set of primers for PCR was designed. The

primers were constructed to provide restriction sites at each end of the coding sequence, as well as in-frame start and stop codons. The sequences of the primers, indicating restriction enzyme cleavage sites and expression landmarks, are shown below.

The paragraph on page 81, lines 11-25 has been amended as follows:

Nucleic acid probes of this invention are those comprising sequences that are complementary or substantially complementary to the MN cDNA sequence shown in ~~Figure 1~~ **Figures 1A-1C** or to other MN gene sequences, such as, the complete genomic sequence of ~~Figure 15 a d~~ **Figures 15A-15F** [SEQ. ID. NO.: 5] and the putative promoter sequence [SEQ. ID. NO.: 27 of Figure 25]. The phrase "substantially complementary" is defined herein to have the meaning as it is well understood in the art and, thus, used in the context of standard hybridization conditions. The stringency of hybridization conditions can be adjusted to control the precision of complementarity. Exemplary are the stringent hybridization conditions used in Examples 11 and 12. Two nucleic acids are, for example, substantially complementary to each other, if they hybridize to each other under such stringent hybridization conditions.

The paragraph on page 83, lines 1-10 has been amended as follows:

However, nucleic acid probes of this invention need not hybridize to a coding region of MN. For example, nucleic acid probes of this invention may hybridize partially or wholly to a non-coding region of the genomic sequence shown in ~~Figure 15a-d~~ **Figures 15A-15F** [SEQ. ID. NO.: 5]. Conventional technology can be used to determine whether fragments of SEQ. ID. NO.: 5 or related nucleic acids are useful to identify MN nucleic acid sequences. [See, for example, Benton and Davis, supra and Fuscoe et al., supra.]

The table on page 84, lines 1-12 has been amended as follows:

Region of Homology within MN Genomic Sequence [SEQ. ID. NO.: 5; ~~Figure 15a-d~~ **Figures 15A-15F**]	SEQ. ID. NOS.	% Homology to Entire Alu-J Sequence
921-1212	59	89.1%
2370-2631	60	78.6%
4587-4880	61	90.1%
6463-6738	62	85.4%
7651-7939	63	91.0%
9020-9317	64	69.8%
		% Homology to One Half of Alu-J Sequence
8301-8405	65	88.8%
10040-10122	66	73.2%.

The paragraph on page 98, lines 9-18 has been amended as follows:

Anti-peptide antibodies are also made by conventional methods in the art as described in European Patent Publication No. 44,710 (published Jan. 27, 1982). Briefly, such anti-peptide antibodies are prepared by selecting a peptide from an MN amino acid sequence as from ~~Figure 1~~ **Figures 1A-1C**, chemically synthesizing it, conjugating it to an appropriate immunogenic protein and injecting it into an appropriate animal, usually a rabbit or a mouse; then, either polyclonal or monoclonal antibodies are made, the latter by a Kohler-Milstein procedure, for example.

The paragraph on page 102, lines 14-19 has been amended as follows:

Mab M75 recognizes both the nonglycosylated GEX-3X-MN fusion protein and native MN protein as expressed in CGL3 cells equally well. Mab M75 was shown by epitope mapping to be reactive with the epitope represented by the amino acid sequence from AA 62 to AA 67 [SEQ. ID. NO.: 10] of the MN protein shown in ~~Figure 1~~ **Figures 1A-1C**.

The paragraph on page 104, lines 1-5 has been amended as follows:

Mab MN9. Monoclonal antibody MN9 (Mab MN9) reacts to the same epitope as Mab M75, represented by the sequence from AA

62 to AA 67 [SEQ. ID. NO.: 10] of the ~~Figure 1~~ **Figures 1A-1C** MN protein. As Mab M75, Mab MN9 recognizes both the GEX-3X-MN fusion protein and native MN protein equally well.

The two paragraphs beginning on page 104, line 14 to page 105, line 10 have been amended as follows:

Mab MN12. Monoclonal antibody MN12 (Mab MN12) is produced by the mouse lymphocytic hybridoma MN 12.2.2 which was deposited under ATCC Designation HB 11647 on June 9, 1994 at the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110-2209 (USA). Antibodies corresponding to Mab MN12 can also be made, analogously to the method outlined above for Mab MN9, by screening a series of antibodies prepared against an MN protein/polypeptide, against the peptide representing the epitope for Mab MN12. That peptide is AA 55 - AA 60 of ~~Figure 1~~ **Figures 1A-1C** [SEQ. ID. NO.: 11]. The Novatope system could also be used to find antibodies specific for said epitope.

Mab MN7. Monoclonal antibody MN7 (Mab MN7) was selected from mabs prepared against nonglycosylated GEX-3X-MN as described above. It recognizes the epitope on MN represented by the amino acid sequence from AA 127 to AA 147 [SEQ. ID. NO.: 12] of the ~~Figure 1~~ **Figures 1A-1C** MN protein. Analogously to methods described above for Mabs MN9 and MN12, mabs corresponding to Mab

MN7 can be prepared by selecting mabs prepared against an MN protein/polypeptide that are reactive with the peptide having SEQ. ID. NO.: 12, or by the stated alternative means.

The paragraph on page 109, lines 1-11 has been amended as follows:

Preferred antisense oligonucleotides according to this invention are gene-specific ODNs or oligonucleotides complementary to the 5' end of MN mRNA. Particularly preferred are the 29-mer ODN1 and 19-mer ODN2 for which the sequences are provided in Example 10, infra. Those antisense ODNs are representative of the many antisense nucleic acid sequences that can function to inhibit MN gene expression. Ones of ordinary skill in the art could determine appropriate antisense nucleic acid sequences, preferably antisense oligonucleotides, from the nucleic acid sequences of ~~Figures 1 and 15a-d~~ **Figures 1A-1C and 15A-15F**.

The paragraph beginning on page 121, line 14 to page 122, line 2 has been amended as follows:

As shown in ~~Figure 6~~ **Figures 6A and 6B** discussed below in Example 5, MX antigen was found to be present in MaTu-infected fibroblasts. In Zavada and Zavadova, supra, it was reported that a p58 band from MX-infected fibroblasts could not be detected by

RIP with rabbit anti-MaTu serum. That serum contains more antibodies to MX than to MN antigen. The discrepancy can be explained by the extremely slow spread of MX in infected cultures. The results reported in Zavada and Zavadova, supra were from fibroblasts tested 6 weeks after infection, whereas the later testing was 4 months after infection. We have found by immunoblots that MX can be first detected in both H/F-N and H/F-T hybrids after 4 weeks, in HeLa cells after six weeks and in fibroblasts only 10 weeks after infection.

The three paragraphs beginning on page 122, line 5 to page 123, line 9 have been amended as follows:

~~Figure 6~~ **Figures 6A and 6B** graphically ~~illustrates~~ **illustrate** the expression of MN- and MX- specific proteins in human fibroblasts, in HeLa cells and in H/F-N and H/F-T hybrid cells, and contrasts the expression in MX-infected and uninfected cells. Cells were infected with MX by co-cultivation with mitomycin C-treated MX-infected HeLa. The infected and uninfected cells were grown for three passages in dense cultures. About four months after infection, the infected cells concurrently with uninfected cells were grown in petri dishes to produce dense monolayers.

A radioimmunoassay was performed directly in confluent petri dish (5 cm) culture of cells, fixed with methanol

essentially as described in Example 3, supra. The monolayers were fixed with methanol and treated with ^{125}I-labeled MAbs M67 (specific for exogenous MX antigen) or M75 (specific for endogenous MN antigen) at 6 x 10^4 cpm/dish. The bound radioactivity was measured; the results are shown in ~~Figure 6~~ **Figures 6A and 6B**.

~~Figure 6 shows~~ **Figures 6A and 6B show** that MX was transmitted to all four cell lines tested, that is, to human embryo fibroblasts, to HeLa and to both H/F-N and H/F-T hybrids; at the same time, all four uninfected counterpart cell lines were MX-negative (top graph of ~~Figure 6~~ **Figures 6A and 6B**). MN antigens are shown to be present in both MX-infected and uninfected HeLa and H/F-T cells, but not in the fibroblasts (bottom graph of ~~Figure 6~~ **Figures 6A and 6B**). No MN antigen was found in the control H/F-N, and only a minimum increase over background of MN antigen was found in MaTu infected H/F-N. Thus, it was found that in the hybrids, expression of MN antigen very strongly correlates with tumorigenicity.

The paragraph on page 129, lines 11-23 has been amended as follows:

Titration of antibodies to MN antigen is shown in ~~Figure 11~~ **Figures 11A and 11B**. Ascitic fluid from a mouse carrying M75 hybridoma cells (A) is shown to have a 50% end-point

at dilution 1:1.4 x 10^{-6}. At the same time, ascitic fluids with MAbs specific for MX protein (M16 and M67) showed no precipitation of ^{125}I-labeled GEX-3X-MN even at dilution 1:200 (result not shown). Normal rabbit serum (C) did not significantly precipitate the MN antigen; rabbit anti-MaTu serum (B), obtained after immunization with live MX-infected HeLa cells, precipitated 7% of radioactive MN protein, when diluted 1:200. The rabbit anti-MaTu serum is shown by immunoblot in Example 4 (above) to precipitate both MX and MN proteins.

The paragraph beginning on page 132, line 15 to page 133, line 11 has been amended as follows:

Ultrathin sections of control and of MX-infected HeLa cells are shown in ~~Figure 13 A-D~~ **Figures 13A-13D**. Those immuno-electron micrographs demonstrate the location of MN antigen in the cells, and in addition, the striking ultrastructural differences between control and MX-infected HeLa. A control HeLa cell (Figure 13A) is shown to have on its surface very little MN antigen, as visualised with gold beads. The cell surface is rather smooth, with only two little protrusions. No mitochondria can be seen in the cytoplasm. In contrast, MX-infected HeLa cells ~~(Figure 13B and C)~~ **(Figures 13B and 13C** show the formation of abundant, dense filamentous protrusions from their surfaces. Most of the MN antigen is located on those filaments, which are

decorated with immunogold. The cytoplasm of MX-infected HeLa contains numerous mitochondria (Figure 13C). Figure 13D demonstrates the location of MN antigen in the nucleus: some of the MN antigen is in nucleoplasm (possibly linked to chromatin), but a higher concentration of the MN antigen is in the nucleoli. Again, the surface of normal HeLa (panels A and E of Figure 13) is rather smooth whereas MX-infected HeLa cells have on their surface, numerous filaments and "blebs". Some of the filaments appear to form bridges connecting them to adjacent cells.

The paragraph on page 151, lines 11-23 has been amended as follows:

The MN-expressing NIH 3T3 cells displayed spindle-shaped morphology, and increased refractility; they were less adherent to the solid support and smaller in size. The control (mock transfected cells) had a flat morphology, similar to parental NIH 3T3 cells. In contrast to the control cells that were aligned and formed a monolayer with an ordered pattern, the cells expressing MN lost the capacity for growth arrest and grew chaotically on top of one another ~~(Figure 22a-d)~~ **(Figures 22A-22D**. Correspondingly, the MN-expressing cells were able to reach significantly higher (more than 2x) saturation densities (Table 4) and were less dependent on growth factors than the control cells ~~(Figure 22g-h)~~ **(Figures 22G and 22H)**.

The three paragraphs beginning on page 153, line 11 to page 154, line 13 has been amended as follows:

Flow cytometric analyses of asynchronous cell populations. For the results shown in ~~Figure 23(a)~~ **Figures 23A-1 and 23A-2**, cells that had been grown in dense culture were plated at 1 x 10^6 cells per 60 mm dish. Four days later, the cells were collected by trypsinization, washed, resuspended in PBS, fixed by dropwise addition of 70% ethanol and stained by propidium iodine solution containing RNase. Analysis was performed by FACStar using DNA cell cycle analysis software [Becton Dickinson; Franklin Lakes, NJ (USA)].

For the analyses shown in ~~Figure 23(b) and (c)~~ **Figures 23B-1, 23B-2 and 23C**, exponentially growing cells were plated at 5 x 10^5 cells per 60 mm dish and analysed as above 2 days later. Forward light scatter was used for the analysis of relative cell sizes. The data were evaluated using Kolmogorov-Smirnov test [Young, J. Histochem. Cytochem., 25: 935 (1977)]. D is the maximum difference between summation curves derived from histograms. D/s(n) is a value which indicates the similarity of the compared curves (it is close to zero when curves are similar).

The flow cytometric analyses revealed that clonal populations constitutively expressing MN protein showed a

decreased percentage of cells in G1 phase and an increased percentage of cells in G2-M phases. Those differences were more striking in cell populations grown throughout three passages in high density cultures ~~[Figure 23(a)]~~ **[Figures 23A-1 and 23A-2]**, than in exponentially growing subconfluent cells ~~[Figure 23(b)]~~ **Figures 23B-1 and 23B-2**. That observation supports the idea that MN protein has the capacity to perturb contact inhibition.